Biogas slurry application alters soil properties, reshapes the soil microbial community, and alleviates root rot of *Panax notoginseng*

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Abstract

Background. *Panax notoginseng* is an important herbal medicine in China, where this crop is cultivated by replanting of seedlings. Root rot disease threatens the sustainability of *P. notoginseng* cultivation. Water flooding (WF) is widely used to control numerous soilborne diseases, and biogas slurry shows positive effects on the soil physiochemical properties and microbial community structure and has the potential to suppress soilborne pathogens. Hence, biogas slurry flooding (BSF) may be an effective approach for alleviating root rot disease of *P. notoginseng*; however, the underlying mechanism needs to be elucidated.

Methods. In this study, we conducted a microcosm experiment to determine if BSF can reduce the abundance of pathogens in soil and alleviate root rot of *P. notoginseng*. Microcosms containing soil collected from a patch of *P. notoginseng* showing symptoms of root rot disease were subjected to WF or BSF at two concentrations for two durations (15 and 30 days), after which the changes in their physicochemical properties were investigated. Culturable microorganisms and the root rot ratio were also estimated. We next compared changes in the microbial community structure of soils under BSF with changes in WF and untreated soils through high-throughput sequencing of bacterial 16S rRNA (16S) and fungal internal transcribed spacer (ITS) genes amplicon.

Results. WF treatment did not obviously change the soil microbiota. In contrast, BSF treatment significantly altered the physicochemical properties and reshaped the bacterial and fungal communities, reduced the relative abundance of potential fungal pathogens (*Fusarium*, *Cylindrocarpon*, *Alternaria*, and *Phoma*) and suppressed culturable fungi and *Fusarium*. The changes in the microbial community structure corresponded to decreased root rot ratios. The mechanisms of fungal pathogen suppression by BSF involved several factors, including by inducing anaerobic/conductive conditions, altering the soil physicochemical properties, enriching the anaerobic and culturable bacteria, and increasing the phylogenetic relatedness of the bacterial community. Overall, BSF promoted the bacterial community and suppressed fungal and pathogenic taxa.

Conclusions. BSF application can reshape the soil microbial community, reduce the abundance of potential pathogens, and alleviate root rot in *P. notoginseng*. Thus, it is a promising practice for controlling root rot disease in *P. notoginseng*.

61 **Keywords** Biogas slurry; *Panax notoginseng*; Root rot disease; Soil physicochemical property;

62 Microbiota;

63

64 **Introduction**

65   Biogas production, through anaerobic digestion of human and animal waste, straw, and other

66 organic materials, has emerged as a potentially important source of renewable energy (Kougias

67 & Angelidaki 2018). In recent decades, the number of biogas plants in China and other countries

68 has greatly increased (Kougias & Angelidaki 2018). The rapid development of biogas production

69 has led to increases in the amount of biogas residue, including that of biogas slurry (BS). BS is a

70 high-quality organic fertilizer with high levels of residual organic carbon, available nitrogen, and

71 other minerals useful for crop growth (Abubaker et al. 2012; Insam et al. 2015). Hence,

72 application of BS alters the soil microbial community structure, increases soil microbial diversity

73 and activity, improves soil quality, and enhances crop yield (Abubaker et al. 2012; Cristina et al.

74 2020; Walsh et al. 2012). Furthermore, BS contains numerous organic compounds [such as

75 volatile fatty acids (VFAs)] and a high concentration of ammonia, which can suppress *Fusarium*

76 spp. and other soil pathogens (Cao et al. 2014; Huang et al. 2015b). Consistent with the effects of

77 other organic amendments, BS application can suppress soilborne pathogens and reduce the

78 incidence of plant diseases (Cao et al. 2016; Insam et al. 2015). For example, Cao et al. (2016)

79 showed that application of BS, under both normal moisture and flooding conditions (BSF),

80 suppressed *Fusarium* wilt disease in watermelon. Appropriate application of BS to soil

81 contributes to the decreased use of pesticides and chemical fertilizers, demonstrating the

82 potential of BS in sustainable agriculture production and environmental protection (Insam et al.

83 2015; Walsh et al. 2012).

84   *Panax notoginseng* (Burk.) F. H. Chen ("Sanqi" in Chinese) is a member of the family

85 *Araliaceae* and is one of the most important herbal medicines in China. Wenshan County in

86 Yunnan province (southwest China) is the geo-authentic habitat for Sanqi planting. Sanqi

87 requires a specific ecological habitat and is grown using a continuous monoculture system,

88 leading to a high prevalence of replanting failure. Therefore, the planting area has been extended

89 to the surrounding areas and beyond, which has further negatively affected the yield and quality

90 of Sanqi and led to the development of more serious diseases, such as mildew and root rot.

91 Abiotic and biotic factors, including the deterioration of soil physicochemical characteristics,

92   nutrient imbalance, soilborne diseases, and accumulation of phytotoxic allelochemical substrates

93   cause replanting failure (Liu et al. 2019; Wu et al. 2008). Root rot is the most common fungal

94   disease associated with soilborne pathogens, such as *Fusarium*, *Alternaria*, *Cylindrocarpon*, and

95   *Phoma* (Li et al. 2020; Miao et al. 2006; Wang et al. 2021). Among these, *Fusarium* spp. are the

96   primary pathogens because of their wide host range and high stress tolerance (e.g., drought and

97   high temperatures), making them difficult to control (Liu et al. 2019). Different measures have

98   been explored for mitigating diseases in Sanqi, among which, rotation (with maize, rape, and

99   wheat) is a preferred practice (Liu et al. 2019; Tang et al. 2020). However, even after 10–20

100 years, rotation cannot completely eliminate diseases because of the long lifespan of pathogens

101 (Tang et al. 2020). Thus, replant failure still occurs in Sanqi cultivation because of the soil is

102 sterilized using fungicides and fumigation (Yang et al. 2015). Additionally, fungicides are not

103 environmentally benign and are gradually becoming unavailable because of the strict regulation

104 of agrochemicals. Hence, more effective and non-chemical biological measures, such as soil

105 flooding and/or addition of organic materials (straw, BS), are urgently needed. Water flooding

106 (WF), an ancient and widely used practice in China and other Asian countries, effectively

107 controls numerous soilborne diseases (Niem et al. 2013). BSF, which incorporates organic

108 materials and associated microorganisms, can increase soil microbial activity, and promote

109 nutrient availability (Cao et al. 2016; Dahunsi et al. 2021). BSF also has positive effects on the

110 soil physiochemical properties (such as increasing the soil pH, available K contents, NH_4^+-N

111 contents, water-soluble carbon, and water-soluble nitrogen) and microbial community structure

112 (suppression of soilborne pathogens), and shows potential for alleviating plant diseases (Cao et

113 al. 2014; Cao et al. 2016). In addition, BSF introduces organic materials into soil (Abubaker et

114 al. 2012; Insam et al. 2015) and rapidly creates reductive/anaerobic conditions (Cao et al. 2016),

115 and is similar to anaerobic soil disinfestation (ASD) by flooding soil after the addition of organic

116 residues (Blok et al. 2000). Because ASD has been widely used for effective disinfestation of

117 various soilborne pathogens (Hewavitharana & Mazzola 2016; Strauss & Kluepfel 2015; Zhou et

118 al. 2019), BSF shows similar application potential.

119   Studies of the changes in the soil physiochemical properties and microbial community under

120 BSF and/or WF treatments for Sanqi root rot disease are lacking, particularly studies aimed at

121 evaluating changes in pathogen abundance. We predicted that compared with WF, BSF can more

122 effectively suppress pathogens and alleviate root rot disease in *P. notoginseng*. Here, we

123 conducted a microcosm experiment using Sanqi root rot soil, which was treated with WF and

124 two concentrations of BSF. We aimed to (1) explore the changes in the physicochemical

125 properties and composition of microbial community in the treated soils, (2) assess the efficacy of

126 WF and BSF in suppressing pathogens and alleviating root rot symptoms, and (3) examine the

127 mechanisms underlying these responses.

128

Materials and Methods

130 **Biogas slurry and soil characteristics**

131 BS was collected from an internal-circulation biogas reactor, using vegetable juice waste as

132 input material, at Bio-energy and Environment Engineering Research Group, Yunnan Normal

133 University, Kunming, China. The reactor had been stably operated for 6 months. The chemical

134 characteristics of the BS were as follows: total solid $1.0 \pm 0.2\%$, chemical oxygen demand 7072

135 ± 65 mg/L, total N 612.2 ± 22.4 mg/L, NH_4^+-N 282.7 ± 14.6 mg/L, NO_3^--N 49.6 ± 5.3 mg/L, and

136 pH 6.5 ± 0.2. Bulk soil samples were collected from a Sanqi plantation in Wenshan (23°40′N,

137 102°35′E, 1400 m altitude), Yunnan Province, China, in January 2019. This site is classified as

138 Latosols based on the Chinese soil taxonomy. The region is characterized by a subtropical

139 climate, with a mean annual precipitation and temperature of 1100–1319 mm and 15–18 °C,

140 respectively. Sanqi has been consecutively cultivated for 5 years and suffered severe root rot

141 disease at this site. *Fusarium* spp. have been frequently isolated and identified as the main

142 pathogens underlying this disease. The chemical characteristics of the initial soil were as follows:

143 pH 6.8 ± 0.2, organic matter 19.5 ± 1.7 g/kg, total N 925.5 ± 43.2 mg/kg, available P 66.0 ± 4.5

144 mg/kg, and available K 94.4 ± 7.1 mg/kg. Approximately 10 kg of continuous-cropping soil was

145 randomly collected from the 0–20 cm soil layer, homogeneously mixed, and sieved through a 2-

146 mm mesh to remove stones and plant debris.

147

148 **Experimental design and soil sampling**

149 A soil microcosm experiment was conducted to investigate the effects of WF and BSF

150 treatments on the soil physicochemical properties and microbiota. Three treatments (1:1 soil:

151 water ratio was selected according to a previous study (Wen et al. 2015)), each with three

152 replicates, were performed in 500-mL microcosms (glass bottles) as follows: (1) water flooding

153 treatment (CK): 250 g soil flooded with 250 mL sterilized water; (2) diluted-BS treatment (CH):

154 250 g soil flooded with 250 mL diluted 50%-concentration BS (equivalent to ~ 0.3 g N/kg soil);

155 and (3) original BS treatment (CF): 250 g soil flooded with 250 mL original BS (equivalent to ~

156 0.6 g N/kg soil). Specifically, after adding BS at two concentrations to the initial soil (untreated

157 soil, named as "Soil"), the homogeneously mixed slurry samples were immediately collected

158 (named as "CH0d" and "CF0d" respectively, representing two BS-flooded soils without

159 incubation), and then the glass bottles were sealed and incubated in the dark at 28 °C. WF

160 treatment was performed using a similar procedure. After incubation for 15 and 30 days, day 15

161 (CK15d, CH15d, and CF15d, representing water and two BS-flooded soils with 15 day's

162 incubation) and day 30 soil samples (CK30d, CH30d, and CF30d, representing water and two

163 BS-flooded soils with 30 day's incubation) were collected (~ 20 g per sample). Thus, a total of

164 27 samples was obtained. Each sample was divided into two parts: one part was used to analyze

165 the soil physicochemical properties and assess the number of culturable bacteria and fungi, and

166 the other was stored at –80 °C for subsequent DNA extraction.

167

168 **Analysis of soil physicochemical properties**

169 Soil pH and oxidation-reduction potential (Eh) were determined using a PHS-3C Meter with

170 the corresponding electrodes (INESA Scientific Instrument Co., Ltd., Shanghai, China) and a

171 1:2.5 soil/water (w/v) suspension. Electrical conductivity (EC) was measured in a 1:5 soil/water

172 (w/v) suspension using a DDS-11A Conductivity Meter (INESA Scientific Instrument Co., Ltd.).

173 Ammonia nitrogen was measured with a continuous flow analyzer (FIAstar TM 5000 System;

174 FOSS, Hilleroed, Denmark). Potential toxic organic acids (mainly VFAs, including acetate,

175 propionate, butyrate, and valerate) were analyzed using gas chromatography on a GC-9790II

176 (FULI Apparatus Co. Ltd., Shanghai, China), as previously described (Zhao et al. 2018). All

177 values were obtained from three replicates in each treatment.

178

179 **Assay of culturable bacteria, fungi, and *Fusarium***

180 To assess the effects of water or BS treatments on the microbial community in the soils, we

181 determined the population densities of culturable bacteria, fungi, and *Fusarium* (a potential

182 pathogen) using a standard 10-fold dilution plating assay. Briefly, the bacteria on nutrient agar

183 (NA) medium plates were counted, and colony-forming units (CFUs) were counted after

184 incubation at 30 °C for 2 days. Fungi and *Fusarium* were enumerated using Martin's Rose

185 Bengal agar and Komada's selective medium (Komada 1975), respectively, after incubation at

186 25 °C for 5 days. All values were obtained from three replicates.

187

Pathogenicity assay of soil on Sanqi root

189 A pathogenicity assay (potential to cause root rot) of the water or BS-treated soils described

190 above was performed on Sanqi roots *in vitro* according to a previous method (Luo et al. 2019)

191 with some modifications. Briefly, on day 30 of the experiment, surface water was removed and

192 the remaining treated soils were air-dried for 4 days (approximately 40% water holding capacity)

193 and then thoroughly mixed. Healthy 1-year-old roots were washed with sterile water, then

194 surface sterilized with 1% sodium hypochlorite for 6 min, and washed 4 times with sterile water.

195 The roots were transferred to a plastic container containing the treated soils and were completely

196 covered with the soils. Each treatment (CK, CH, and CF) and the untreated soil contained three

197 replicates (each with 10 roots). All treatments were randomly placed in an incubator at 25 °C and

198 watered every 3 days to maintain the water holding capacity at approximately 40%. After 30

199 days, the root rot ratio (%) was calculated as the number of roots showing rot-rooted symptoms

200 divided by the total number of tested roots.

201

Soil DNA extraction and sequencing

203 Total genomic DNA was extracted from each sample using a PowerSoil® DNA Isolation Kit

204 (MoBio Laboratories, Carlsbad, CA, USA). The DNA concentration and quantity were evaluated

205 using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA,

206 USA); the extracted DNA was stored at –20 °C until use. Marker genes, amplified using

207 polymerase chain reaction (PCR), were sequenced to characterize the community composition

208 and diversity of bacteria and fungi. The prokaryotic 16S V3-V4 and fungal internal transcribed

209 spacer (ITS2) regions were amplified using the primer pairs 341F

210 (CCTAYGGGRBGCASCAG)/ 806R (GGACTACNNGGGTATCTAAT) and ITS3-

211 2024F(GCATCGATGAAGAACGCAGC)/ ITS4-2409R (TCCTCCGCTTATTGATATGC),

212 respectively (Takahashi et al. 2014; Toju et al. 2012). PCR was performed using Phusion® High-

213 Fidelity PCR Master Mix (New England Biolabs, Ipswich, MA, USA). The amplification

214 conditions were as follows: initial denaturation at 95 ℃ for 2 min, followed by 25 cycles of

215 denaturation at 95 ℃ for 30 s, annealing at 55 ℃ for 30 s, and extension at 72 ℃ for 1 min, with

216 a final extension at 72 °C for 10 min. The PCR products were purified with a GeneJET™ Gel

217 Extraction Kit (Thermo Fisher Scientific) and used to construct sequencing libraries using an Ion

218 Plus Fragment Library Kit (Thermo Fisher Scientific), according to the manufacturer's

219 recommendations. Library quality was assessed using a Qubit@ 2.0 Fluorometer (Thermo Fisher

220 Scientific). The library was sequenced on an Ion S5™ XL platform and 600 bp single-end reads

221 were generated. The sequencing data generated was deposited in the NCBI Sequence Read

222 Archive database (accession numbers PRJNA661430 and PRJNA661668 for the bacterial and

223 fungal sequences, respectively).

224

225 **Bioinformatics analyses**

226 Raw sequencing reads were filtered and analyzed using the QIIME software (v1.9.1)

227 (Caporaso et al. 2010). Briefly, primer sequences and low quality reads with scores below Q30

228 were filtered, and chimeras were detected using Chimera UCHIME. Filtered high-quality

229 sequences showing ≥ 97% similarity were clustered and assigned to the same operational

230 taxonomic unit (OTU) using Uparse software (v7.0.1001) (Edgar 2013). To obtain taxonomic

231 information on the bacterial and fungal OTUs, a representative sequence of each OTU was

232 generated and aligned against the Silva (v132) and Unite (v7.2) databases, respectively. The

233 OTU abundance was normalized using a standard sequence number corresponding to each

234 sample with the fewest sequences (45,000 sequences for bacteria and 32,000 for fungi).

235 Alpha diversity indices, including the observed-species and Shannon indices, were

236 determined to analyze within-sample diversity. Beta diversity analyses were performed to

237 evaluate differences between samples (treatments). Principal coordinate analysis (PCoA) and

238 hierarchical cluster analysis were both based on Bray-Curtis distance using an OTU abundance

239 table, and then visualized using "ggplot2" (v3.3.3) and "ggtree" (v2.2.1) (Yu et al. 2017) in R

240 (v3.5.1), respectively. Linear discriminant analysis (LDA) coupled with effect size was

241 performed using LEfSe software (Segata et al. 2011), and the default LDA score was 3.5.

242 Metastats analysis was performed using permutation tests between groups at the genus level to

243 obtain the *P* values (adjusted with the "false discovery rate" method) (Ouzounis et al. 2009).

244 The bacterial and fungal community functions were predicted using FAPROTAX (Louca et

245 al. 2016) and FunGuild (Nguyen et al. 2016) software, respectively. The nearest taxon index

246 (NTI) was calculated for the microbial phylogenetic diversity using the null model independent

247 swap with 999 randomization runs and 1000 iterations, utilizing "Picante" package (v1.8.1)

248 (Stegen et al. 2012) in R (v3.5.1).

249

250 **Statistical analysis**

251 To determine the significance of differences in microbial community composition between

252 groups, non-parametric multivariate analysis of variance (PERMANOVA, transformed data by

253 Bray-Curtis, permutation = 999) was performed using the adonis function of "vegan" package

254 (v2.5-7) in R (v3.5.1). The Spearman's correlations among microbial genera, environmental

255 factors, putative pathogens, and the most abundant bacteria/fungi were analyzed with base

256 functions in R (v3.5.1). A Mantel test (999 permutations) was applied to calculate the correlation

257 between environmental factors and the microbial community using "vegan" (v2.5-7). Regression

258 analyses were performed to investigate the relationships between the root rot ratio and

259 biotic/abiotic factors (culturable microorganisms, soil properties, and alpha/beta-diversity

260 indices). Statistical differences between treatments (soil properties, alpha diversity, NTI values)

261 were calculated using a one-way analysis of variance followed by a Tukey's HSD test ($P < 0.05$

262 was considered to indicate significant differences).

263

264 # Results

265 **Physicochemical properties of soil**

266 To evaluate changes in the physicochemical properties of continuous-cropping soil under

267 WF and BSF treatments, VFAs, pH, Eh, EC, and NH_4^+-N were determined (Fig. 1). Addition of

268 BS (CH0d-CF0d) increased the acetate and propionate contents in the initial soil ("Soil"). Total

269 VFAs were significantly higher in the BS-addition groups (t-test with $P < 0.05$: CH0d 282 ± 10

270 vs. Soil 7 ± 1; CF0d 582 ± 82 vs. Soil 7 ± 1). The levels of acetate and propionate gradually

271 decreased to below the limits of detection (CH30d and CF30d) after 30 days, whereas butyrate

272 levels increased and valerate was maintained at nearly constant levels.

273 Following WF and BSF treatment for 15 or 30 days, pH and NH_4^+-N increased and Eh

274 decreased as compared to in the initial soil (Fig. 1, CK15d or CK30 vs. Soil; CH15d or CH30 vs.

275 Soil; CF15d or CF30 vs. Soil). These changes were significant in the BSF treatments ($P < 0.05$),

276 which induced more conductive/anaerobic conditions (Eh < 0 mV) compared to those in the WF

277 treatment. Additionally, compared with those in the corresponding CK, pH and EC were higher,

278   whereas Eh was lower in BSF-treated soils. This trend was more obvious at higher

279   concentrations of BS (CF treatment, $P < 0.05$) after a longer treatment duration (Eh value: -68

280   mV in CH15d, -105 mV in CF15d; -88 mV in CH30d, -112 mV in CF30d).

281

Pathogenicity assay of soil

283   Pathogenicity (i.e., potential to cause root rot) of water or BS-treated soils on Sanqi roots

284   was assayed, and the root rot ratio was calculated to evaluate the effects of different treatments.

285   Symptoms of root rot typically included soft, necrotic, and brown roots (Fig. 2a), whereas health

286   roots showed no symptoms and remained intact (Fig. 2b). Results indicated that both untreated

287   continuous-cropping ("Soil") and water-flooded ("CK") soil showed the highest pathogenicity

288   potential, as approximately 90% of the root showed root rot symptoms (Fig. 2c). In contrast, BSF

289   treatment (CH and CF) significantly decreased ($P < 0.05$) the root rot ratios to as low as 10–20%

290   (Fig. 2c).

291

Population of culturable microorganisms in soil

293   As shown in Fig. 2d, compared to in the untreated soil ("Soil"), 15 days of BSF treatment

294   suppressed the population of culturable microorganisms, with a significant decrease in the fungal

295   and *Fusarium* populations ($P < 0.05$). After 30 days, the fungal and *Fusarium* population further

296   decreased slightly whereas the bacterial population restored to its original level. On day 30, the

297   number of fungi was the lowest in CF, and the smallest *Fusarium* population was observed in

298   CH. Moreover, the ratios of *Fusarium*/fungi and bacteria/fungi were both significantly reduced

299   by BSF treatment, with CH treatment showing the lowest *Fusarium*/fungi ratio. In contrast,

300   compared with untreated soil, water-flooded treatment (CK) had no obvious effects on the

301   culturable microorganisms tested ($P < 0.05$).

302

Alpha diversity of microbial community

304   Next, we evaluated the effects of WF and BSF treatments on the microbial community based

305   on sequencing of the bacterial 16S and fungal ITS marker genes. After quality control, the

306   sequences were clustered into 4984 and 1123 OTUs for bacteria and fungi, respectively. Alpha

307   diversity analysis showed that the bacteria and fungi exhibited similar trends in the observed-

308   species/Shannon indices after BSF (CH15d, CF15d, CH30d, and CF30d) and WF (CK15d and

309   CK30d) treatments (Table 1). Specifically, the Shannon indices of both CH0d and CF0d

310   decreased significantly compared with those in the original soil ($P < 0.05$). After incubation for

311   15 or 30 days, there was a notable increase in both indices in BSF-treated soils compared to in

312   CH0d (or CF0d), although the values were lower than their corresponding CK values after the

313   same duration. Additionally, both indices of diluted-BSF (CH, CH15d and CH30d) were slightly

314   higher than those in the original BSF (CF, CF15d and CF30d) after the same treatment duration.

315

Structure of microbial community

317   PCoA and hierarchical cluster analysis were applied to compare the dissimilarities and

318   hierarchical clustering of the microbial community between treatments, respectively. For

319   bacteria, PERMANOVA showed a significant difference among all groups ($R^2 = 0.89$, $P =$

320   0.001), and PCoA ordination demonstrated that the first two axes accounted for 81% of the

321   variation (Fig. 3a). All samples were mainly separated into three clusters: "Soil-CK15d-CK30d"

322   and "CH0d-CF0d" samples were separately clustered; the remaining BSF-treated samples

323   (CH15d, CH30d, CF15d, and CF30d) formed the third cluster, in which "CH15d-CH30d" (CH,

324   diluted-BS treated samples) and "CF15d-CF30d" (CF, initial BS-treated samples) were slightly

325   separated along the first axis, consistent with the results of hierarchical cluster analysis (Fig. 3c).

326   A similar clustering pattern was found for fungi (PERMANOVA, $R^2 = 0.81$, $P = 0.001$), but

327   BSF-treated samples were more dispersed than the corresponding bacterial samples (Fig. 3b, d).

328   Based on the different soil treatments and sample-clustering modes, the samples were combined

329   to five larger groups for comparative analysis: (1) Soil (representing initial soil); (2) CHCF0d

330   (CH0d and CF0d, representing BSF soils without incubation); (3) CK15.30d (CK15d and

331   CK30d, representing WF soils incubated for 15 and 30 days); (4) CH15.30d (CH15d and CH30d,

332   representing 50% diluted-BSF soils incubated for 15 and 30 days); and (5) CF15.30d (CF15d

333   and CF30d, representing original BSF soils incubated for 15 and 30 days). To assess the

334   statistical significance of differences in the bacterial and fungal communities between the five

335   groups, differences between pairwise groups were examined using PERMANOVA, which

336   revealed significant differences between groups, except for in "Soil vs. CK15.30d" (Table S1,

337   bacteria: $R^2 = 0.45$, $P = 0.062$; fungi: $R^2 = 0.27$, $P = 0.053$).

338

Composition of fungal community

All fungal OTUs were classified into 10 phyla and 190 genera. Of these, one OTU (OUT_1, only classified at the kingdom level, Dataset S1) that was not present in the initial soil showed a notably high abundance in CH0d (63%) and CF0d (66%) compared to in the other groups. After incubation for 15 or 30 days, the abundance of OTU_1 declined to 10% in CH15.30d and 14% in CF15.30d. Overall, BSF significantly modulated the composition of the fungal community from the phylum to the genus levels. Specifically, *Ascomycota* and *Basidiomycota* could be classified across all samples (Fig. 4a). *Ascomycota* were highly enriched in Soil and CK15.30d (67–75%) but were depleted in CH15.30d (11–15%, metastats analysis with $P < 0.05$). At the genus level, six genera that presented average relative abundances above 1% were *Fusarium*, *Chaetomium*, *Staphylotrichum*, *Setophoma*, *Humicola*, and *Saitozyma*. Of these, *Fusarium* was dominant across all samples (Fig. 4b) and was significantly depleted in CH15.30d soils compared with that in CK15.30d and CF15.30d soils ($P < 0.05$). The other five genera were significantly decreased after BSF treatment ($P < 0.05$). LEfSe analysis indicated that some *Ascomycota* affiliations (*Sordariales*, *Staphylotrichum*, *Setophoma*, and *Fusarium*) declined markedly in CH15.30d compared with those in CK15.30d soils ($P < 0.05$; Fig. 5f).

Furthermore, 219 fungal OTUs were common to both Soil and CHCF0d or unique to CHCF0d (Fig. 5a). According to previous studies (Li et al. 2020; Liu et al. 2019; Miao et al. 2006), combined with the taxa detected in our study, four taxa (*Fusarium*, *Cylindrocarpon*, *Alternaria*, and *Phoma*) were potential fungal pathogens of Sanqi root rot. Next, we analyzed changes in the relative abundance of these fungi in response to the BSF and/or WF treatments (Fig. 5c). Compared with those in the initial soil, the levels of these four genera decreased by 4–200-fold after BSF treatment (CH15.30d and CF15.30d), except for a slight increase in *Fusarium* in the CF15.30d group (21.169% in Soil, 28.429% in CF15.30d). In contrast, the abundance of *Fusarium*, *Cylindrocarpon*, and *Phoma* was slightly decreased in WF soils (CK15.30d) compared to in the initial soil.

Composition of bacterial community

Prokaryotic OTUs were classified into two kingdoms (Archaea and Bacteria), 64 phyla, and 608 genera. The most abundant Archaea OTU (phylum *Euryarchaeota*; genus *Methanocorpusculum*) was present in CF30 (0.2%). The five dominant phyla, which accounted

370   for 82% of the bacterial community across all samples, were *Proteobacteria*, *Bacteroidetes*,

371   *Firmicutes*, *Synergistetes*, and *Acidobacteria* (Fig. 4c). After BSF treatment, the relative

372   abundance of *Proteobacteria*, *Actinobacteria*, and *Acidobacteria* decreased (from 46% to 18–

373   40%, 11% to 0.4–2%, and from 10% to 0.5–2%, respectively), whereas that of *Firmicutes*,

374   *Synergistetes*, and *Bacteroidetes* increased significantly ($P < 0.05$; from 0.9% to 10–16%, from

375   0.04% to 3–14%, and from 11% to 22–49%, respectively). At the genus level, the top 10 genera

376   present at more than 1% abundance were *unidentified Rikenellaceae*, *Trichococcus*,

377   *Macellibacteroides*, *Lactobacillus*, *Arcobacter*, *unidentified Synergistaceae*, *Proteiniphilum*,

378   *Sphingomonas*, *Pseudomonas*, and *Bacteroides* (Fig. 4d). LEfSe analysis showed that the phyla

379   *Firmicutes* (affiliated order *Clostridiales*; family *Ruminococcaceae*; genus *Anaerovorax*),

380   *Synergistetes* (genus *unidentified Synergistaceae*), and *Bacteroidetes* (genus *unidentified*

381   *Rikenellaceae*, *Macellibacteroides*, and *Proteiniphilum*) were consistently and significantly

382   enriched following BSF treatment ($P < 0.05$). *Actinobacteria* (genus *Bryobacter*), *Proteobacteria*

383   (genus *Sphingobium* and *unidentified GammaProteobacteria*), and *Gemmatimonadetes* (family

384   *unidentified Gemmatimonadaceae*) were the marker taxa (identified by LEfSe) in the initial soil

385   (Fig. 5e). Among the top genera, *unidentified Rikenellaceae*, *Macellibacteroides*, *Lactobacillus*,

386   *unidentified Synergistaceae*, *Proteiniphilum*, *Pseudomonas*, and *Sedimentibacter* were specific to

387   the CH0d and CF0d groups compared to in the initial soil. Furthermore, the abundance of some

388   bacterial genera differed significantly between groups; the relative abundance of *Trichococcus*,

389   *Lactobacillus,* and *Arcobacter* was significantly higher in CH0d-CF0d than that in other groups

390   ($P < 0.05$). Additionally, there were no significant differences between the initial soil and

391   CK15.30d at either the phylum or genus level (Fig. 4c, d).

392   Comparing the bacterial OTUs between the initial Soil and CHCF0d soils revealed that 466

393   OTUs were unique to CHCF0d (Fig. 5b). These OTUs are mainly facultative or anaerobic

394   bacteria (*unidentified Rikenellaceae*, *unidentified Synergistaceae*, and *Sedimentibacter*).

395

396   NTI analysis of the microbial community

397   NTI analysis of the bacterial and fungal samples revealed that all NTI values were higher

398   than zero (Fig. 5d), and the average NTI value (3.22 ± 0.78) of the bacterial community was

399   significantly higher ($P < 0.05$) than that of fungi (1.91 ± 0.58), providing evidence for

400   phylogenetic assembly, particularly in bacteria. After BS amendment (CH0d and CF0d), the

401 bacterial NTI decreased significantly compared with that in the initial soil ($P < 0.05$). The

402 bacterial NTI of CH0d increased after 15 days (CH15d) and continued to increase after 30 days

403 (CH30d), whereas no significant changes were observed under the original BSF or CK treatment.

404 Furthermore, the NTI of the CH treatments was higher than that of the corresponding CF

405 treatment, particularly after 30 days. A similar trend was observed for the fungal NTI, except that

406 the NTI of CH30d was lower than that of CH15d.

407

408 **Function analysis of microbial community**

409 The functions of the bacterial OTUs were annotated using FAPROTAX. As shown in Fig.

410 S1a, all BSF-treated samples harbored enriched functions involved in anaerobic metabolism,

411 such as "methanogenesis", "sulfate_respiration", and "methanotrophy", which was consistent

412 with the increased abundance of anaerobic bacteria (e.g., *Methanosaeta*, and *Desulfovibrio*).

413 Fungal functional prediction using FunGuild showed that functions related to "plant

414 pathogen" were more enriched in the Soil and CK15.30d samples than in the CH15.30 and

415 CF15.30d samples (Fig. S1b).

416

417 **Correlation between the potential pathogens and top abundant taxa**

418 Spearman's correlation analysis between the four potential pathogens and most abundant

419 (top 20) bacteria, as well as fungi at the genus level revealed that the four pathogens were

420 significantly negatively correlated with most bacteria; however, they were significantly

421 positively correlated with most fungi (Fig. 6a, b). In addition, correlation analysis between the

422 top 25 abundant bacteria and fungi revealed a higher proportion of negative (71%) than positive

423 (29%) correlations (Fig. 6c).

424

425 **Correlation between microbial community and soil physicochemical properties**

426 As indigenous microbiota is shaped by soil condition (Liu et al. 2019), we used Eh, and EC,

427 pH, NH_4^+-N, and VFAs as environmental factors to clarify the relationships between the

428 microbiota and soil properties. The Mantel test showed that environmental factors were

429 significantly correlated with changes in the bacteria (r = 0.55, P = 0.001) and fungi (r = 0.42, P =

430 0.002). In addition, Spearman's rank correlation analysis was used to evaluate the correlations

431 between soil properties and bacteria or fungi at the genus level. Among the top 25 genera, most

432   bacteria were significantly negatively correlated with Eh ($P < 0.05$) but positively correlated with

433   EC, NH_4^+-N, and VFAs (acetate, propionate, and valerate, Fig. 6c).

434   Most fungi were significantly negatively correlated with EC, pH, NH_4^+-N, and VFAs ($P <$

435   0.05), and positively correlated with Eh. Particularly, the potential pathogens (*Fusarium*,

436   *Cylindrocarpon*, and *Phoma*) were significantly negatively correlated with EC, pH, NH_4^+-N, and

437   all tested VFAs ($P < 0.05$).

438   Additionally, variation partitioning analysis (Fig. S2) revealed that the soil physicochemical

439   properties, treatment modes (BS application or not), and treatment days explained 79% and 69%

440   of the observed variation in the bacterial and fungal compositions, respectively. Soil properties,

441   which were clearly affected by the treatment mode, explained 20% and 31% of the observed

442   variation in the bacterial and fungal compositions, respectively, whereas treatment days only

443   explained a small portion of the variation (2% and 3%, respectively).

444

445   **Correlation between root rot ratio and biotic/abiotic factors**

446   Linear regression analyses (Fig. S3) showed that the root rot ratio was significantly ($P <$

447   0.05) positively correlated with the number of culturable fungi and *Fusarium*, ratio of *Fusarium*

448   to fungi, ratio of fungi to bacteria, alpha diversity (Shannon index) of the bacterial and fungal

449   communities, and Eh value in the soil but negatively correlated with the number of culturable

450   bacteria ($P = 0.16$), concentrations of total VFAs ($P = 0.056$), EC ($P < 0.05$), and NH_4^+-N ($P <$

451   0.05). Moreover, the root rot ratio was significantly correlated with the bacterial and fungal beta-

452   diversity index (PCoA1) ($P < 0.05$).

453

454   **Discussion**

455   Maintaining soil fertility and controlling soilborne diseases are vital for sustainable crop

456   production. BS, an organic fertilizer with high quality and biological activity, is an excellent

457   candidate for green agriculture (Cao et al. 2016; Insam et al. 2015; Walsh et al. 2012). We

458   investigated the impacts of BSF application on the microbial community (including potential

459   pathogens) and occurrence of root rot symptoms in Sanqi continuous soil.

460

461   **BSF improved soil conditions**

462   BSF has similar characteristics as ASD; both introduce organic materials into soil and create

463   reductive/anaerobic conditions (i.e., in this study, mesocosms were flooded and sealed to reduce

464   oxygen availability). Mechanisms underlying the suppression and efficacy of ASD include

465   strong anaerobic/reductive conditions, changes in the microbial population, and production of

466   organic acids and ammonia (Huang et al. 2015b; Momma et al. 2011). ASD was recently

467   adopted to control Sanqi soil disease and alleviate replant failure (Li et al. 2019). Considering the

468   procedures used to apply these agents, the mechanisms through which ASD suppresses

469   pathogens may also be partially applicable to BSF. The present study showed that *Firmicutes*-

470   affiliated anaerobes (*Clostridiales* and *Ruminococcaceae*, frequently enriched in response to

471   ASD treatment), which produce toxic VFAs (Huang et al. 2015a; Momma et al. 2013; Mowlick

472   et al. 2013), were significantly enriched after BSF treatment. High concentrations of VFAs can

473   suppress pathogens in ASD-treated soil (Momma et al. 2006). In the present study, both BS

474   treatments incorporated a large amount of BS-derived VFAs (acetate and propionate) into the

475   initial soil, and the persistence of butyrate generated in the soils showed the potential to suppress

476   pathogens throughout the experiment. This result was supported by the negative correlation

477   between pathogens and VFAs. Notably, many facultative and anaerobic taxa (such as

478   *unidentified Rikenellaceae*, *Trichococcus*, *Macellibacteroides*, and *Lactobacillus*), which were

479   unique to CHCH0d and derived from BS, were incorporated into the initial soil by BSF.

480   Furthermore, some of these taxa were highly abundant throughout BSF treatment (30 days).

481   These taxa, in turn, consume residual oxygen and create more anaerobic conditions, further

482   suppressing pathogens (Wen et al. 2015). The present study showed that most fungi (including

483   three potential pathogens) were positively correlated with the Eh value, indicating they were

484   suppressed by the anaerobic/reductive conditions. This prediction is consistent with the

485   observation that most fungi cannot grow under anaerobic conditions (Takaya 2002).

486   Furthermore, the increased pH and NH_4^+-N from BSF may mitigate soil acidification in the

487   Sanqi cropping system. Therefore, soil pre-conditioned with BSF presented better global

488   physicochemical properties and higher quality than the initial soil, which may contribute to

489   synergistic inactivation of pathogens. However, BSF of soil is not always practical, particularly

490   in hilly mountain areas. Hence, irrigating soil with BS to 100% or less of the water holding

491   capacity, similar to previous studies (Cao et al. 2016; Wen et al. 2015), may be an alternative

492   approach for suppressing pathogens; the efficacy of this method in the field requires further

493   study.

494   In contrast, compared with those in the BSF-treated soils or initial soil, no significant
495   changes in either the bacterial or fungal communities were observed in the WF treatment.
496   Furthermore, there was no obvious suppression of potential pathogens, including the culturable
497   *Fusarium*, or a reduction in the root rot ratio. This result is inconsistent with those of previous
498   studies showing that WF can effectively control pathogens (Kelman & Cook 1977; Niem et al.
499   2013). Thus, WF may not lead to significant changes in the microbiota or suppression of
500   pathogens, emphasizing the key role of anaerobic microorganisms and organic matter derived
501   from BS.

502

503   **BSF reshaped the soil microbial community to a relatively pathogen-suppressive state**

504   BSF reshaped the bacterial and fungal community to form a different community structure
505   (BSF-treated groups clustered together, as shown in Fig. 3), which was consistent with the
506   results of a previous study (Cao et al. 2016). Importantly, BSF treatment reduced the abundance
507   of potential pathogens (*Fusarium*, *Cylindrocarpon*, *Alternaria*, and *Phoma*), which might be the
508   key factor for reducing the root rot ratio of Sanqi. *Fusarium*, one of the main pathogens, was
509   significantly reduced to a similar number by both CH and CF treatments according to the plate
510   count assay (Fig. 2d). However, ITS sequencing showed that relative abundance of *Fusarium*
511   was reduced following CH treatment but increased following CF treatment (Fig. 4b). Indeed,
512   differences exist between culturable and culture-independent sequencing methods. The former is
513   mainly affected by number of culturable microorganisms detected in a specific medium, the
514   latter is primarily affected by the DNA extraction method, primers used to amplify the 16S or
515   ITS genes, and sequence data analysis method (Bonk et al. 2018). We counted the number of
516   *Fusarium* using Komada's selective medium, which has been used in previous studies (Tao et al.
517   2020; Wen et al. 2015); Tao et al. (2020) showed that quantitative colony counting was
518   positively correlated with the results of qPCR. One possible reason for this is that CF treatment
519   induced more anaerobic/reductive conditions (lower Eh value) compared to CH, suppressing
520   more total fungi, whereas *Fusarium* which has a higher tolerance was less affected (Ebihara &
521   Uematsu 2014), leading to an increased ratio of *Fusarium* to fungi in CF compared with that in
522   CH (Fig. 2d). The suppression of other fungi, except for *Fusarium*, was greater following CF,
523   resulting in a higher relative abundance of *Fusarium* in CF than in CH or Soil (Fig. 2d). The root
524   rot ratio was positively correlated with the culturable *Fusarium* and ratio of *Fusarium*/fungi,

525 supporting that a reduced *Fusarium*/fungi ratio is conducive to the growth of Sanqi (Zhao et al.
526 2017). Notably, taxa classified based on OTUs are generally accurate at the genus level, and
527 some non-pathogenic *Fusarium* spp. contribute to the abundance but not to the occurrence of
528 disease. As only culturable fungi can be enumerated using the plate count method, further
529 absolute quantification methods, such as qPCR can be applied to verify the results. Additionally,
530 we observed a slightly negative correlation between the root rot ratio and culturable bacteria
531 number, which is consistent with a previous study showing that total number of culturable
532 bacteria is important for disease suppression (Bonanomi et al. 2010).

533 Overall, although potential pathogens in the soils have not been completely eliminated, they
534 were suppressed to a lower level by other fungi or bacteria under BSF treatment, with the
535 potential to form a relative pathogen-suppressive state (Cook 2014). This result was further
536 supported by the functional prediction of fungi, which showed fewer "plant pathogen" and
537 pathogenic potential in BSF-treated soils.

538

539 **BSF shifted bacterial community to harbor more beneficial taxa**

540 Compared with the initial soil, BSF treatment significantly altered the bacterial community:
541 *Synergistetes*, *Firmicutes*, and *Bacteroidetes* were enriched, whereas *Actinobacteria* and
542 *Proteobacteria* were depleted. Members of *Synergistetes* can participate in synergistic acetate
543 oxidation and organic acids digestion to produce substrates for hydrogen methanogens, which
544 play important roles in anaerobic metabolism (Acs et al. 2015). *Bacteroidetes* are beneficial taxa
545 that are abundant in the rhizosphere of wild plants; in contrast, members of *Actinobacteria* and
546 *Proteobacteria* are frequently enriched in soils with pathogen infection and long-term N fertilizer
547 application, and are considered as marker taxa of poor soil quality (Dai et al. 2018; Perez-
548 Jaramillo et al. 2018; Wu et al. 2016). A similar study showed that taxa of *Proteobacteria* and
549 *Actinobacteria* are highly enriched in Sanqi monoculture soil (Zhao et al. 2017). Furthermore,
550 Wu et al. (2016) considered *Proteobacteria* as a marker taxon in Sanqi root rot soil. The
551 decreased abundance of *Proteobacteria* and *Actinobacteria* may reflect the positive effect of
552 BSF treatment and elevated soil micro-conditions against plant disease.

553 Under BSF treatment, the most enriched genera (e.g., *unidentified Rikenellaceae*,
554 *unidentified Synergistaceae,* and *Sedimentibacter*) were mostly facultative/anaerobic. A previous
555 study showed that application of BS to soil caused a change from active microbes to slow

556 growing anaerobes (Chen et al. 2012), which is consistent with the our results. Highly abundant
557 anaerobic taxa have also been observed in maize-Sanqi rotation systems (Zhao et al. 2017),
558 representing improved soil quality against disease. Furthermore, archaeal microorganisms
559 (*Methanocorpusculum* and *Methanosaeta*), although present at relatively low abundance, were
560 enhanced. These facultative/anaerobic taxa may compete with pathogenic fungi for niches that
561 are unfavorable for their survival.

562

563 **Bacterial community was vital for defending against pathogens**

564   Plant roots interact with many microorganisms, including bacteria, fungi, and oomycetes in
565 soil, where fungi and oomycetes can typically cause serious disease, but bacterial community is
566 often negatively correlated with eukaryotic microbes and is vital for plant survival by defending
567 against harmful root related eukaryotes (Duran et al. 2018; Luo et al. 2020). Similarly, we
568 demonstrated that the culturable bacteria were slightly increased but culturable fungi were
569 significantly suppressed under BSF treatment at day 30, similar to a previous study (Walsh et al.
570 2012). Moreover, Spearman's correlation analysis revealed greater negative correlations between
571 the top 25 abundant bacteria and fungi, as well as significantly negative correlations between
572 numerous bacteria and potential fungal pathogens. This indicates a potentially competitive or
573 antagonistic relationship between these two groups. Thus, combined with the negative
574 correlation between the number of culturable bacteria and root rot ratio, we predicted that the
575 bacterial community plays a vital role in suppressing fungal pathogens and controlling disease.
576 However, this study was conducted in microcosms, without considering the interactions between
577 plant roots and microbiota in the field. Pathogen levels may re-increase following planting,
578 possibly because of the stimulation of specific root exudates (Li et al. 2014; Liu et al. 2018).
579 Considering that pathogen resurgence is a risk after planting, this issue may be mitigated by
580 introducing antagonistic bacteria alongside BS application (Yin et al. 2021).

581

582 **Culturable microorganisms can be used as indicators of soil pathogenicity**

583   The root rot ratio was positively correlated with the alpha diversity (Shannon index) of the
584 bacterial and fungal communities. Higher microbial diversity is vital for community stability and
585 pathogen suppression (van Elsas et al. 2012). However, this association is controversial;
586 microbial diversity, e.g., relative abundance, may not be a credible indicator of plant health

587   (Huang et al. 2019; Xiong et al. 2017). In some cases, a community with higher microbial

588   diversity can harbor a lower biomass (i.e., the absolute number of microbes in a certain

589   environment) (Chen et al. 2017), which is consistent with the present study, as shown in the

590   initial soil vs. CH0d (or CF0d) (harboring more microorganisms from BS). In the present study,

591   the highly abundant fungus OUT_1 in CH0d and CF0d caused unevenness in the community

592   and, as expected, led to a decrease in the alpha diversity indices. Alternatively, some absolute

593   quantification measures, such as the counts of culturable microorganisms in this study or

594   combined with qPCR (Tao et al. 2020), may partially reflect the real diversity of the community

595   and serve as a credible indicator of disease suppression.

596

Conclusions

598   We created a conceptual graph describing the effects of BSF application on soil properties

599   and the microbial community structure (Fig. 7). The anaerobic/reductive environments created

600   by BSF treatment elevated the soil physicochemical properties (e.g., pH, NH_4^+-N, and Eh) and

601   strongly influenced the bacterial community. Following BSF treatment, bacteria were enriched

602   and fungi were suppressed. In addition, the phylogenetic relatedness of the bacterial community

603   was higher than that of fungi community (NTI analysis). Together, these factors were

604   responsible for assembling a stable bacterial community that negatively interacted with the

605   fungal community, possibly through antagonism or competition for nutrients and niches, thereby

606   suppressing the fungal pathogens and alleviating root rot. This study provides a valuable

607   reference for the application of BS, which may contribute to alleviating root rot disease in Sanqi

608   production.

609

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614

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824

Alpha diversity of bacterial and fungal communities.

* Values (means ± SD: calculated with three replicates in each group) within the same column followed by different lowercase letters indicate significant differences among different treatments at $P < 0.05$ according to Tukey's HSD test;

Group abbreviations: Soil: initial soil, untreated; CH0d: 50% diluted-BSF without incubation; CF0d: original BSF soils without incubation; CK15d: WF soils incubated for 15 days; CH15d: 50% diluted-BSF soils incubated for 15 days; CF15d: original BSF soils incubated for 15 days ; CK30d: WF soils incubated for 30 days; CH30d: 50% diluted-BSF soils incubated for 30 days; CF30d: original BSF soils incubated for 30 days;

Group	Bacteria		Fungi	
	Observed-species	Shannon	Observed-species	Shannon
Soil	2544 ± 63 a*	9.49 ± 0.13 a	615 ± 48 a	6.49 ± 0.12 a
CH0d	1256 ± 171 c	5.62 ± 0.36 c	250 ± 56 d	2.47 ± 0.32 c
CF0d	1186 ± 41 c	5.76 ± 0.18 c	215 ± 25 d	2.28 ± 0.15 c
CK15d	2357 ± 257 ab	9.26 ± 0.14 a	437 ± 15 bc	6.02 ± 0.16 a
CH15d	1650 ± 128 bc	7.62 ± 0.10 b	287 ± 98 cd	4.39 ± 1.10 b
CF15d	1416 ± 106 c	7.12 ± 0.21 b	206 ± 8 d	3.58 ± 0.22 bc
CK30d	2274 ± 393 ab	9.28 ± 0.44 a	489 ± 41 ab	6.00 ± 0.06 a
CH30d	1441 ± 170 c	7.52 ± 0.28 b	258 ± 59 d	4.18 ± 0.86 b
CF30d	1176 ± 391 c	6.59 ± 0.88 bc	257 ± 67 d	3.85 ± 0.01 bc

Figure 1

Physicochemical properties of soil.

Colors from steel blue to orange represent the lowest to highest value (means ± SD: calculated with three replicates in each group) in each row, and values within the same row followed by different lowercase letters indicate significant differences among different treatments at $P < 0.05$ according to Tukey's HSD test. "ND" means below the limits of detection.

The names of treatment (group) abbreviation are defined in Table 1.

Soil	CH0d	CF0d	CK15d	CH15d	CF15d	CK30d	CH30d	CF30d		
ND	212.03 ± 8.42 b	438.39 ± 63.61 a	ND	ND	82.01 ± 6.67 c	ND	ND	ND	-Acetate	(mg/kg)
ND	55.04 ± 2.98 c	113.43 ± 14.94 a	ND	1.52 ± 2.64 d	72.41 ± 2.14 b	ND	ND	ND	-Propionate	(mg/kg)
ND	5.78 ± 0.22 b	11.52 ± 1.17 b	112.38 ± 6.32 a	111.58 ± 1.33 a	107.28 ± 5.37 a	116.37 ± 3.42 a	107.93 ± 9.76 a	116.8 ± 4.96 a	-Butyrate	(mg/kg)
6.5 ± 0.76 b	8.43 ± 0.37 ab	11.87 ± 3.86 a	8.04 ± 0.37ab	10.39 ± 2.28 ab	9.06 ± 0.31 ab	8.88 ± 0.2 ab	9.32 ± 1.19 ab	9.9 ± 0.17 ab	-Valerate	(mg/kg)
ND	ND	1.66 ± 2.88 a	ND	2.64 ± 2.29 a	1.25 ± 2.17 a	ND	ND	ND	-Isobutyrate	(mg/kg)
ND	1.5 ± 1.3 cd	5.46 ± 3.44 bc	ND	6.14 ± 2.53 b	11.08 ± 1.39 a	ND	ND	ND	-Isovalerate	(mg/kg)
6.83 ± 0.1 c	6.58 ± 0.07 d	6.44 ± 0.06 d	6.84 ± 0.13 c	7.71 ± 0.07 a	7.28 ± 0.04 b	6.9 ± 0.07 c	7.74 ± 0.05 a	7.28 ± 0.04 b	-pH	
0.57 ± 0.12 de	2.48 ± 0.16 b	3.59 ± 0.31 a	0.62 ± 0.01 de	1.3 ± 0.11 c	2.45 ± 0.04 b	0.52 ± 0.01 e	0.92 ± 0.02 d	1.63 ± 0.01 d	-EC	(mS/cm)
98.67 ± 11.5 a	92.33 ± 4.04 a	86.33 ± 4.73 a	-52.67 ± 9.02 b	-68 ± 6 bc	-105.33 ± 9.24 de	-60 ± 8 b	-88 ± 7.21 cd	-112 ± 6 e	-Eh	(mV)
50 ± 5 g	151.67 ± 7.64 d	285 ± 5 a	109 ± 3.61 e	127 ± 3 e	198 ± 6.56 bc	88 ± 7.55 f	181.67 ± 10.41 c	213 ± 8.89 b	-NH$_4^+$-N	(mg/kg)

Figure 2

Sanqi root rot ratio and number of culturable microorganisms in different soils.

(a) Rotted-symptom roots. (b) Healthy roots. (c) Root rot ratio in the initial soil (Soil), WF treated (CK), and BSF treated soils (CH and CF represent 50% diluted- and original BSF soils, respectively). (d) Number of culturable bacteria, fungi, *Fusarium*, and ratio of fungi/bacteria, ratio of *Fusarium*/fungi (up to down). Different lowercase letters indicate significant differences among different treatments at $P < 0.05$ according to Tukey's HSD test.

The names of treatment (group) abbreviation are defined in Table 1.
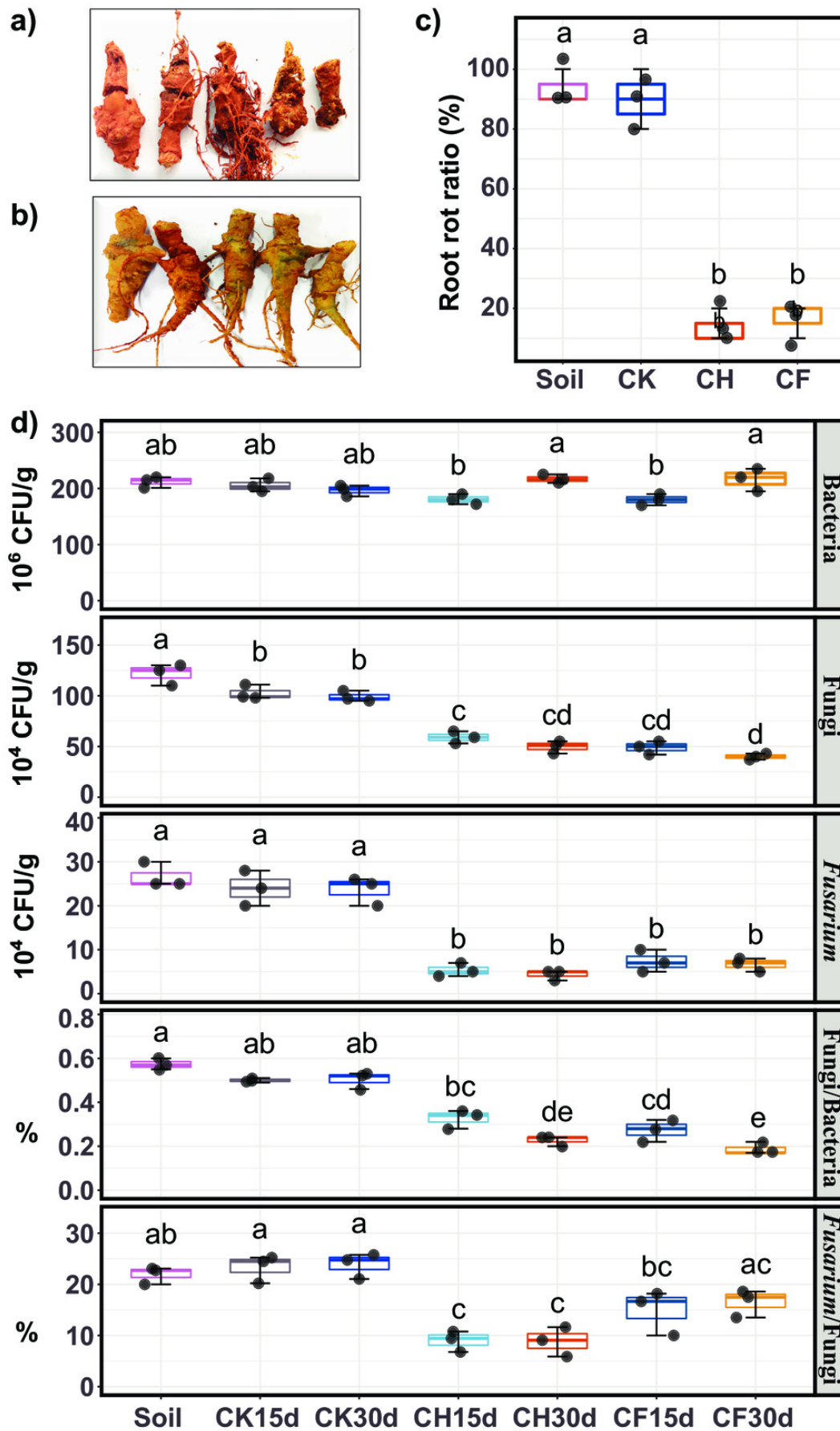
Figure 3

Principle coordinated analysis (PCoA) and hierarchical cluster analysis.

(a) Bacterial PCoA. (b) Fungal PCoA. (c) Hierarchical cluster analysis of bacteria. (d) Hierarchical cluster analysis of fungi. Both analyses are based on Bray-Curtis distance matrix. The dotted lines in a and b indicate the distribution of samples in each group (Soil, CHCF0d, CK15.30d, CH15.30d, or CF15.30d).

Group name stands for: Soil: initial soil, untreated; CHCF0d: BSF soils without incubation; CK15.30d: WF soils incubated for 15 and 30 days; CH15.30d: 50% diluted-BSF soils incubated for 15 and 30 days; CF15.30d: original BSF soils incubated for 15 and 30 days.
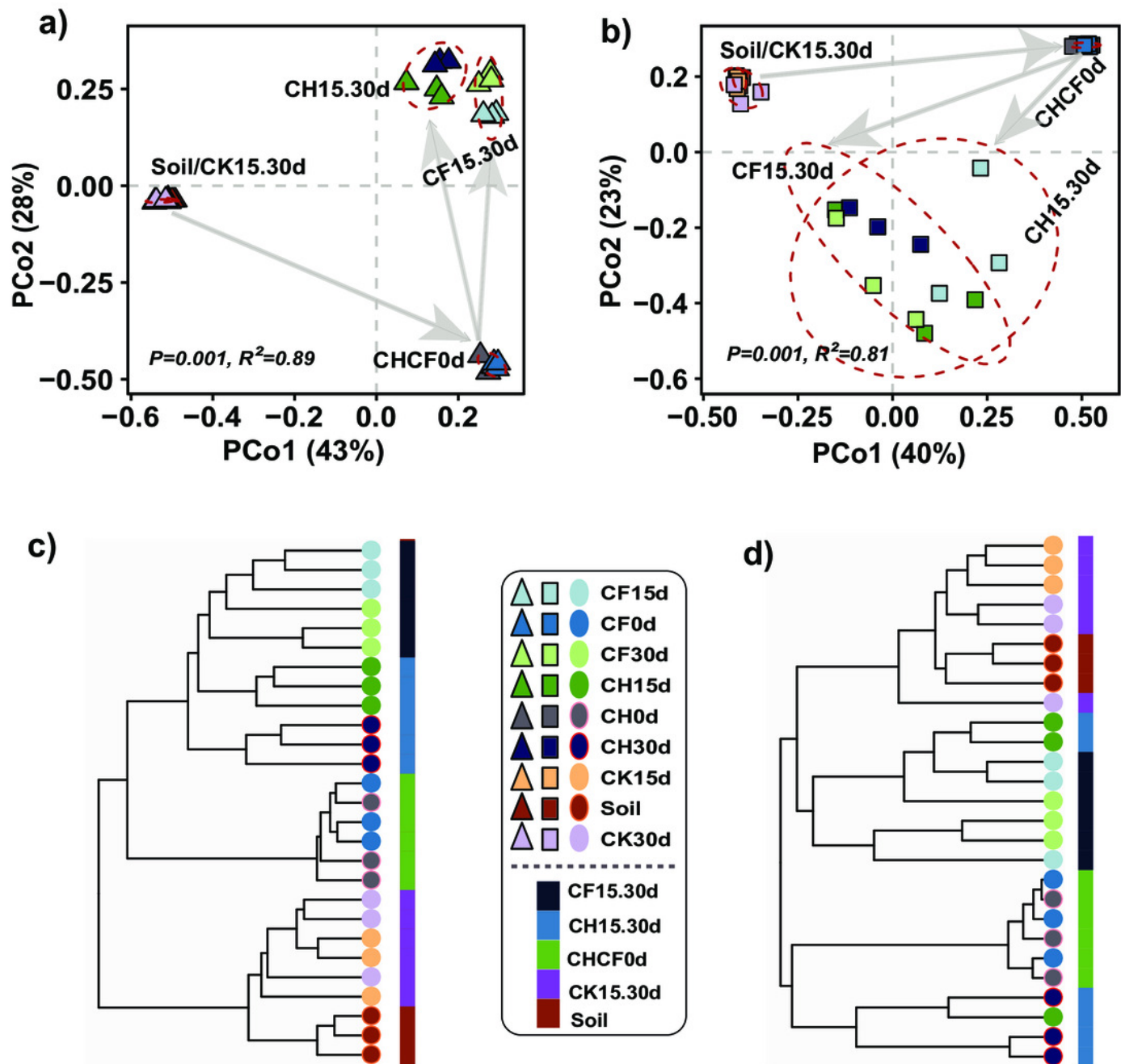
Figure 4

Taxonomic composition of the fungi and bacteria in different treatments.

(a) Fungi at the phylum level. (b) Fungi at the genus level. (c) Bacteria at the phylum level. (d) Bacteria at the genus level. Each bar represents the average relative abundance of a taxon in a treatment. "Others" in a and c represents the sums of the remaining fungal or bacterial phylum except for the top 10 abundant phylum, and "Others" in b and d represents the sums of the remaining fungal or bacterial genus except for the top 20 abundant genus. "*unidentified*" means unclassified, which was followed by the closest taxon at a particular classification level.

The names of treatment (group) abbreviation are defined in Table 1 and Fig. 3.
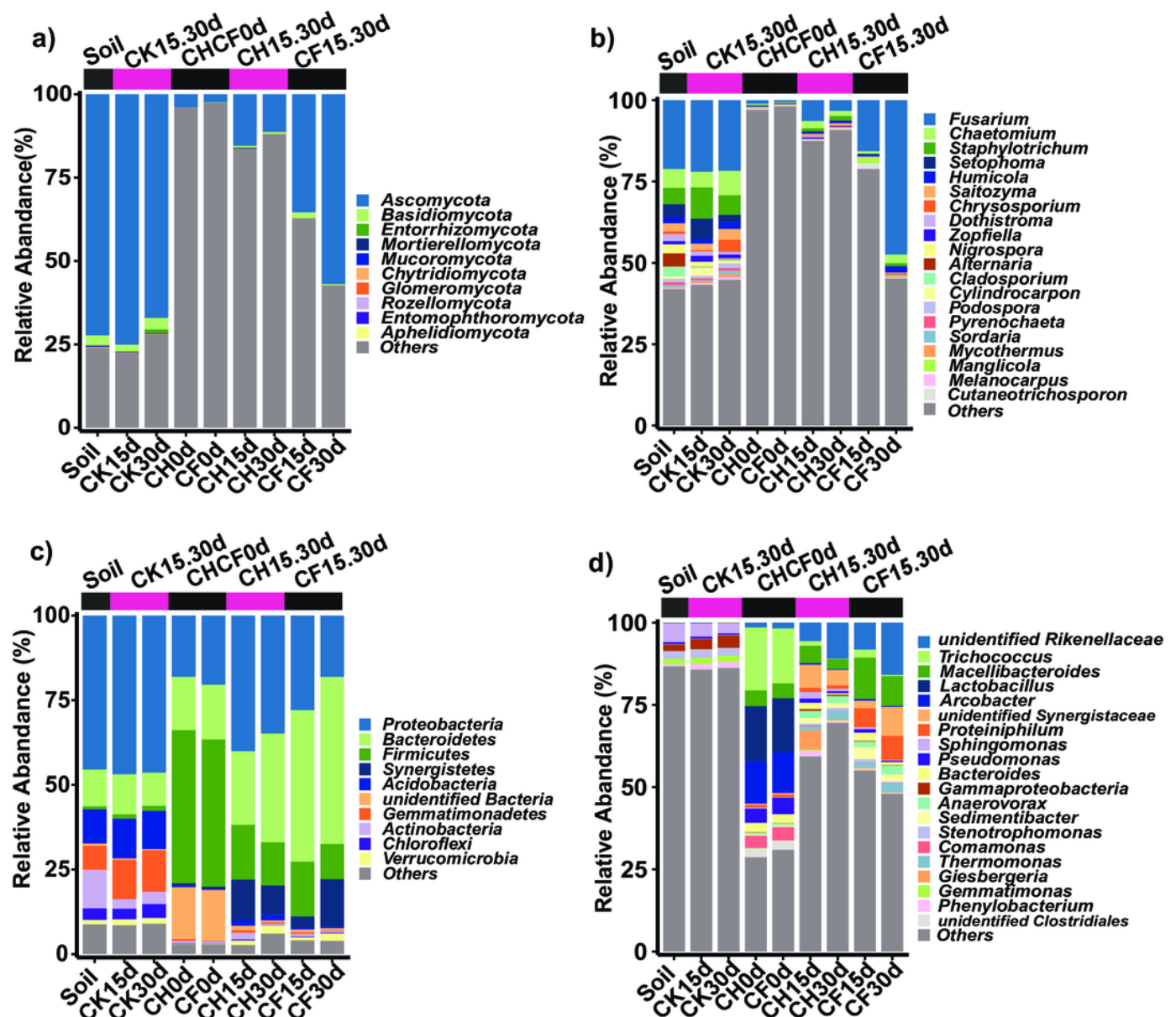
Figure 5

Comparison of the microbial compositon and structure in different treatments.

(a, b) Venn diagrams display the number of unique and shared OTUs between the initial soil and CHCH0d in the fungal (a) and bacterial (b) communities. (c) Comparison of the relative abundance of the four potential fungal pathogens, the point size and number represent the average relative abundance (%). (d) Nearest taxon index (NTI) analysis, different letters indicate significant differences among groups at $P < 0.05$ according to Tukey's HSD test. (e, f) LDA effect size analysis (LEfSe) among different microbial groups, the group-enriched taxa are displayed using ternary diagram.

The names of treatment (group) abbreviation are defined in Table 1 and Fig. 3.
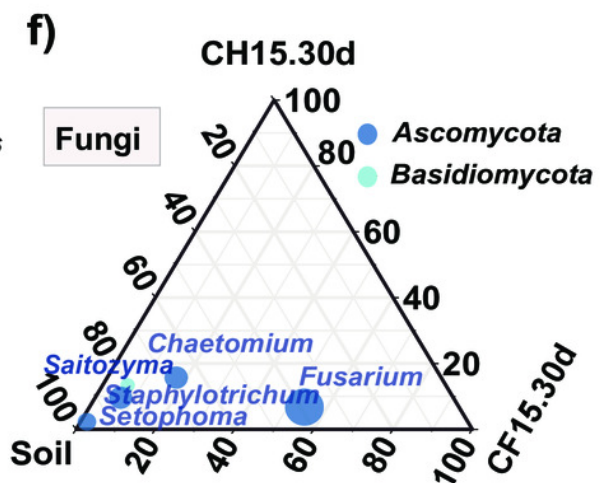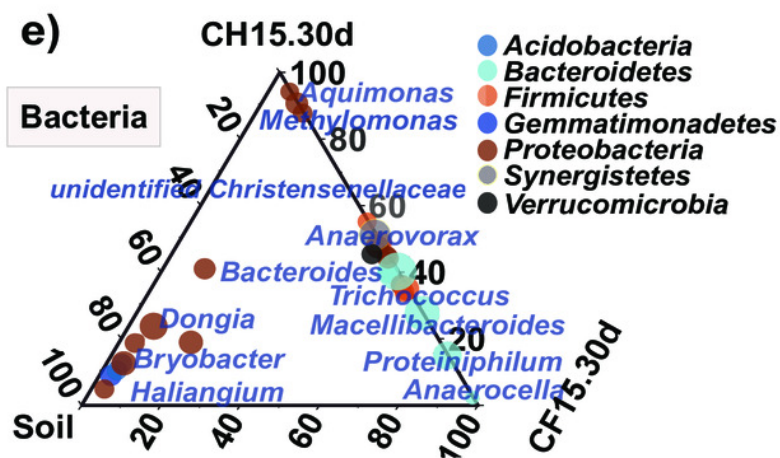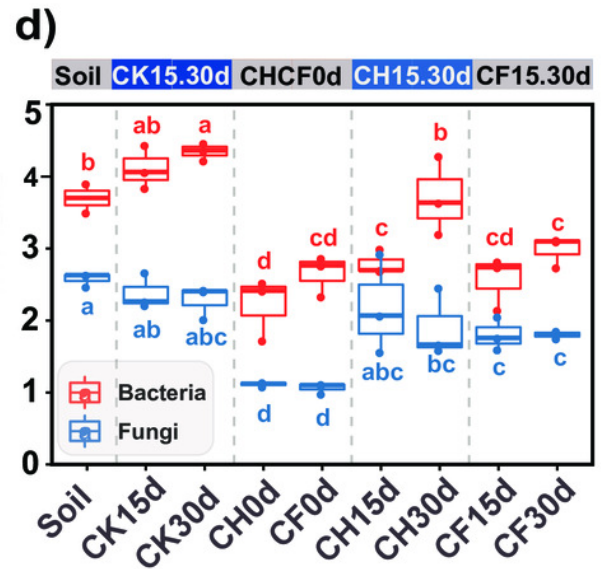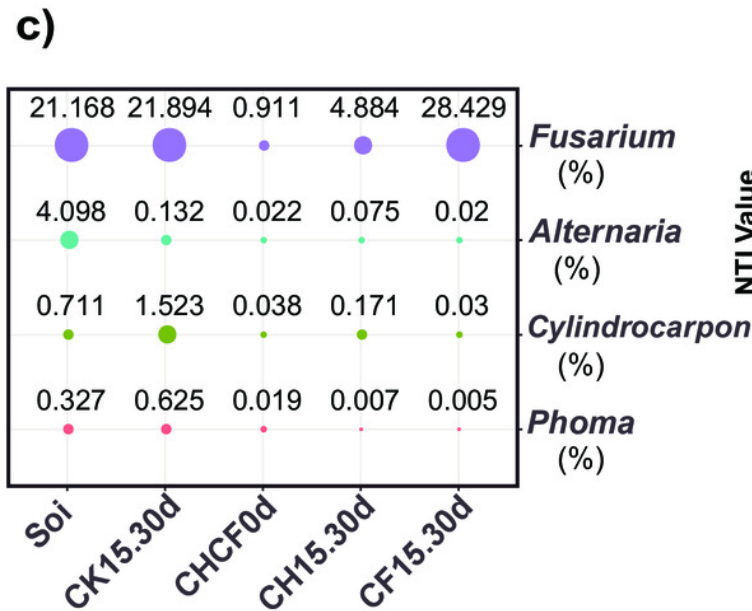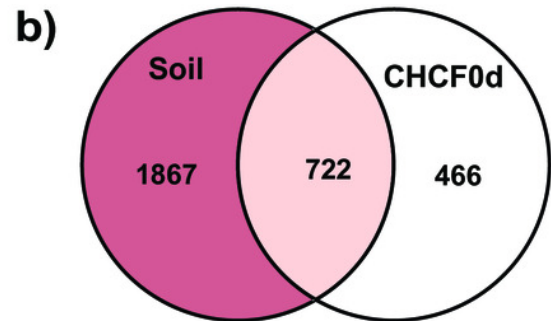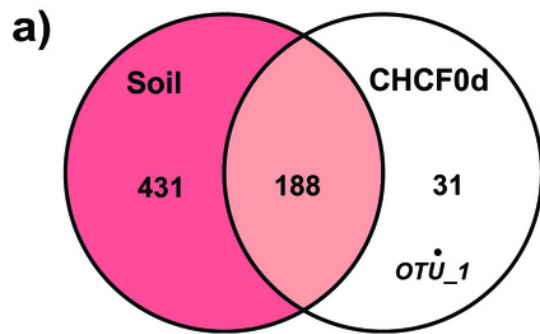
Figure 6

Spearman's correlations between the top bacteria/fungi and potential pathogens, and with the environmental factors.

(a, b) The Spearman's correlations between the top bacteria (a) or fungi (b) and the potential pathogens. (c) The Spearman's correlation between the top bacteria and fungi, and with the environmental factors. The legend value (with corresponding colors from steel blue to red) is the correlation coefficient, the asterisks (* and **) indicate significant correlations at $P < 0.05$ (and 0.01) according to Tukey's HSD test. The colors of links between fungi/bacteria and environmental factors represent significantly ($P < 0.05$) negative (blue) or positive (brown) correlations.
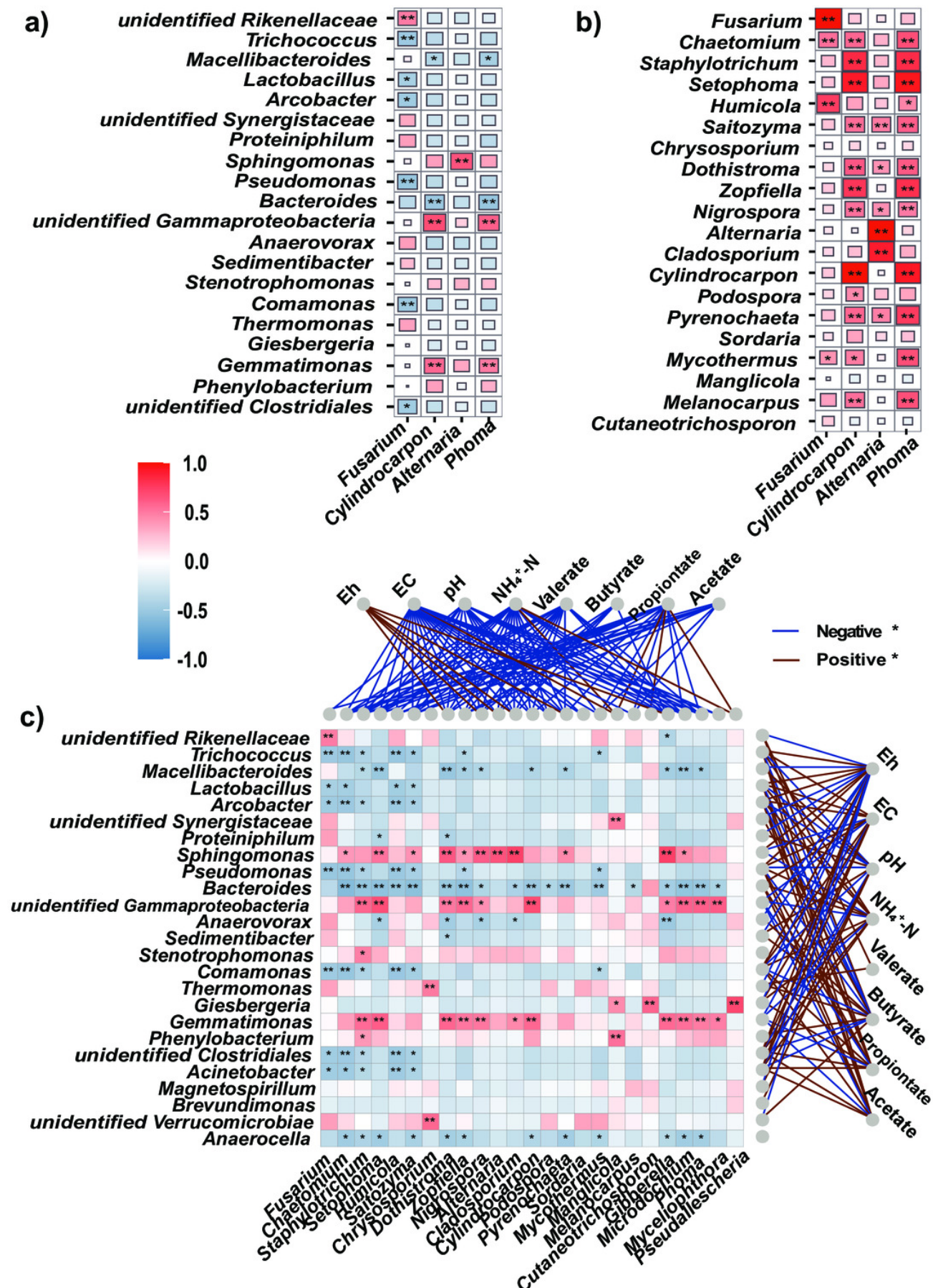
Figure 7

Conceptual graph describing the effects of BS application on soil properties and the microbial community structure (including potential fungal pathogens).